EFFECT OF L-TRP ON PERFORMANCE OF IAA PRODUCING RHIZOBACTERIA ON GROWTH OF SWEETPOTATO (IPOMOEA BATATAS L.)

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Abstrak
Penelitian tentang penambahan L-Triptophan (TRP) pada rhizobakteri penghasil IAA terhadap tanaman ubi jalar yang merupakan percobaan pot bertujuan untuk melihat pengaruh dari L-TRP dan inokulasi isolat rhizobakteri pada pertumbuhan dan hasil ubi jalar, populasi bakteri dan jamur tanah serta kandungan nutrien pada tanah pasir bekas galian tambang timah di Malaysia. Penelitian ini merupakan percobaan pot yang disusun secara Acak lengkap (RAL) yang menggunakan tiga isolat rhizobakteri penghasil IAA yang juga berasal dari perakaran ubi jalar. Dari penelitian ini didapat bahwa isolat rhizobakteri SPR 66 mampu meningkatkan berat kering bahagian atas, kandungan klorofil dan jumlah luas daun, sedangkan isolat SPR 100 mempengaruhi berat kering dan volume akar, pembentukan ubi, kandungan nutrien tanaman dan nutrien tanah. Pemberian L-TRP kepada semua isolat terpilih cendrung meningkatkan pembesaran dan hasil tanaman ubi jalar. Peningkatan ini berkaitan dengan peningkatan keadaan kimia dan aktivitas mikroorganisme di dalam tanah.

Key words: L-Tryptophan, precursors, phytohormones, rhizobacteria

INTRODUCTION
The problem in sweetpotato cultivation is yield reduction with continuous planting, which can be attributed to several factors such as soil degradation, pathogenic effects, and allelopathic effects. There are several factors which affect formation of storage roots of sweetpotato. Phytohormone has been shown to stimulate root initiation on all plants (Frankenberger and Arshad, 1995) and storage root formation on root crops (Ewing, 1987; Arteca, 1996). The endogenous and exogeneous phytohormones found in tuber of some root crops include cytokinins, (Forsline and Langille, 1975), auxins such as Indole-3-acetic acid (IAA) (Melis and van Staden, 1984), salicylic acids, gibberellins and abscissic acid (Ewing, 1987).

IAA is recognized as one of the metabolic products of various microorganisms in the presence of precursor peptone or amino acid tryptophan (TRP) (Reinecke and Bandurski, 1987).

Numerous bacteria and fungi are capable of producing IAA in culture medium. Production of auxins varied greatly among species and strains of the same genus and is also influenced by culture conditions, 1995). The bacterial species which have been known as IAA producer with L-TRP addition were Pseudomonas florcescens, P. putida, and Frankia spp. Other bacteria which are able to produce IAA with and/or without precursor of TRP, include Rhizobium, Azotobacter, Azospirillum, and several Pseudomonas spp.

The potential of utilizing the IAA producing rhizobacterial in enhancing growth of sweetpotato has to be assessed further. Addition of precursor, tryptophan could probably increase the IAA production by the isolates.

The aimed of this study is to determine the effect of L-TRP addition and rhizobacterial inoculation on growth and yield of sweetpotato, the population of bacteria and fungi and the nutrient contents of sandy soil.

MATERIALS AND METHODS
Preparation of Rhizobacterial Inoculant
Three of the rhizobacterial isolates, SPR 66, SPR 83 and SPR 100 that showed positive effect on sweetpotato growth were used. One loopful of each bacterial colony was cultured in separate 250-mL conical flask containing 100mL sterilized King’s
growth B stage and availability of substrate such as amino acid (Frankenberger and Arshad,broth. The flasks were then incubated on a shaker at 100 rpm at 30 °C for 24 hours. The growth of each rhizobacterial culture was measured by determining the optical density at 600 nm using a spectrophotometer.

**Soil Preparation and Treatment**

The sandy loam tailings from an examining area in UPM Malaysia was used. The soil was air-dried and sieved through 0.5 mm sieve, and 10-kg of soil was then placed into plastic bags. Two controls used were Control 1; non-inoculated without IAA and Control 2; non-inoculated with addition of 45 μg/g IAA. Six inoculation treatments used were 1) SPR(sweet potato rhizobacteria) 66, 2) SPR 83, 3) SPR 100, 4) SPR 66+tryptophan (TRP), 5) SPR 83+TRP and 6) SPR 100+TRP. The experimental units were arranged in a Randomised Complete Design (CRD) with four replications.

Cuttings of sweetpotato shoot var. Gendut (with 8 nodes) were cleaned using sterilized water and were dipped into 10 mL of King B broth containing approximately 10^5 cfu mL^-1 rhizobacteria for 15–20 minutes. One inoculated plant cutting was then planted into each plastic pot containing 10 kg unsterilized sandy loam soil.

Chemical fertilizer was given at the rate of 100 kg N ha^-1, 80 kg P ha^-1, and 120 kg K2O ha^-1 and micronutrients. Fertilizers were applied to soil one day before planting. One week after planting, 5-mL of the respective rhizobacterial cultures were inoculated at the base of each plant and the inoculant was applied for 8 weeks. The L-TRP at the rate of 5.3 g/kg was supplied for treatment T6, T7 and T8 one week after planting.

Plants were watered daily and sprayed with insecticides. Plants were harvested at 110 days after planting. At harvest, shoots were cut 1 cm above ground level. The roots were carefully freed from soil and washed clear of adhering soil particles. Fresh weight, size and number of storage root were recorded using the Minolta SPAD-502 502 chlorophyll meter, The SPAD value was converted to standard curve of chlorophyll (y=0.0006x + 0.009). The fresh leaves of each plant were removed from branches for the total leaf area measurements.

The plant shoots and fibrous roots were dried in an oven at 50-60 °C for a week and the plant tissue were then ground (2 mm mesh) and used for the analysis of N, P, K, Ca and Mg contents. Soil samples was air dried and analysed for pH (H2O), organic C, C/N ratio, total N, available P, K, Ca, Mg and IAA contents.

**Soil Microbial Population**

The total population of bacteria and fungi in soil was determined by using the dilution and drop plate technique. The 4.5 g fresh soil was diluted into 95 mL sterilized distilled water. The solution was diluted into 9 mL sterilized distilled water until 10^-5. 20 μL of the last dilution was pipetted and dropped onto plate containing King’s B agar for bacteria and Potato Dextrose Agar for fungi.

All data were subjected to Analysis of variance (ANOVA). Means were compared using the honestly significant difference according to Tukey’s Studentized Range (HSD) test.

**Results and Discussion**

**Effects of Rhizobacterial Inoculation and L-TRP Addition on Growth of Sweetpotato**

The results showed that dry weights of shoot and fibrous root (Figure 1a) and volume of root (Figure 1b) of sweetpotato plants were not significantly affected by rhizobacterial isolate and L-TRP addition.

This shows that plant dry weight and root volume was not influenced by the rhizobacterial inoculation and by the addition of precursor. However, non-inoculated plant without IAA supplied seemed to have lower plant growth.

**Plant and Soil Analysis**

The chlorophyll content of leaf was measured at one week before harvest by formation was significantly (p ≤ 0.05) affected by rhizobacterial inoculation.
and L-TRP addition. However, the number of storage root was not significantly influenced by rhizobacterial isolate and addition of TRP (Table 1).

The results showed that the storage root initiation and formation of sweetpotato plant were influenced by rhizobacterial inoculation and TRP addition, but number of storage roots was not significantly different. Addition of TRP on isolate SPR 100 has the high value on fresh weight of storage root compared to other plants. The non-inoculated plants without IAA supplied did not form the storage roots. However, addition of 45 g/mL IAA into non-inoculated (Control 2) could stimulate formation of storage roots.

Rhizobacterial inoculation and TRP addition significantly (p≤0.05) influenced the chlorophyll content and total leaf area of sweetpotato (Figure 2). The chlorophyll content of non-inoculated plant without IAA (Control1) was significantly (p ≤ 0.05) lower than inoculated plant and un-inoculated plant supplied with IAA (Control2). However, the chlorophyll contents of plant were not significantly different between three rhizobacterial isolates (Figure 2a).

Rhizobacterial inoculation significantly (p≤0.05) stimulated sweetpotato total leaf area (Figure 2b). Plant inoculated with SPR 83+TRP showed high value on total leaf area. There was no significant difference in total leaf area between three bacterial isolates and L-TRP addition. The results

Figure 1. Effect of Rhizobacterial Inoculation and L-TRP Addition on Shoot and Root Dry Weights (a) and Root Volume (b) of Sweetpotato (NS means not significant different at p< 0.05 according to Tukey’s studentized range test)

Table 1. Effect of rhizobacterial inoculation and TRP addition to storage root formation of sweetpotato plants

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Number</th>
<th>Fresh Weight (g)</th>
<th>Length (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control (2)</td>
<td>2.0</td>
<td>8.68 b</td>
<td>10.21</td>
</tr>
<tr>
<td>SPR 66</td>
<td>1.0</td>
<td>6.00 b</td>
<td>8.00</td>
</tr>
<tr>
<td>SPR 83</td>
<td>1.0</td>
<td>7.41 b</td>
<td>8.75</td>
</tr>
<tr>
<td>SPR 100</td>
<td>1.0</td>
<td>14.70 ab</td>
<td>10.00</td>
</tr>
<tr>
<td>SPR 66 +TRP</td>
<td>1.0</td>
<td>14.45 ab</td>
<td>11.88</td>
</tr>
<tr>
<td>SPR 83+TRP</td>
<td>1.0</td>
<td>17.90 a</td>
<td>11.88</td>
</tr>
<tr>
<td>SPR 100+TRP</td>
<td>1.5</td>
<td>25.67 a</td>
<td>13.83</td>
</tr>
<tr>
<td>HSD_{(0.05)}</td>
<td>2.1 (NS)</td>
<td>15.29</td>
<td></td>
</tr>
</tbody>
</table>
indicated that the chlorophyll and total leaf area in soil added with 45 \( \text{g mL}^{-1} \) IAA paralleled that of rhizobacterial inoculation.

Rhizobacterial inoculation and addition of L-TRP into sweetpotato plant significantly \((p \leq 0.05)\) increased concentration of N, P and K in tissue, but not significantly effected concentration of Ca and Mg (Table 2).

Concentration of N in tissue was significantly higher in plants inoculated with SPR 100 with L-TRP addition than the non-inoculated plant without IAA (Control 1). No significant difference was observed between non-inoculated (Control 2) and other inoculated treatment. The concentration of N in tissue ranged between 2.71 to 3.49%.

Plant inoculated with SPR 83 showed the highest concentration of P in tissue and was significantly \((p \leq 0.05)\) higher than Control 1. However, no significant difference was observed between Control 2 and other inoculation treatments. The concentration of P in tissue ranged between 0.42-0.67%.

The highest concentration of K in tissue was observed in the non-inoculated plant without IAA (Control 1). Rhizobacterial inoculation and tryptophan addition did not increase the K concentration in tissue and the concentration of K ranged between 3.35

\[\text{HSD}(0.05) = 542.85\]

**Figure 2.** Effects of Rhizobacterial Inoculation and L-TRP Addition on (a) Chlorophyll Content and (b) Total Leaf Area of Sweetpotato Tissue. Mean values with the same letter were not significantly different at \(p \leq 0.05\) according to Tukey’s studentized range (HSD) test.

**Table 2: Effect of rhizobacterial inoculation and L-TRP addition on nutrient concentration of sweetpotato shoots**

<table>
<thead>
<tr>
<th>Treatments</th>
<th>N</th>
<th>P</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1)-IAA</td>
<td>2.71</td>
<td>0.42</td>
<td>6.51</td>
<td>2.46</td>
<td>0.23</td>
</tr>
<tr>
<td>Control (2)+IAA</td>
<td>2.94</td>
<td>0.55</td>
<td>4.77</td>
<td>2.62</td>
<td>0.28</td>
</tr>
<tr>
<td>SPR 66</td>
<td>3.18</td>
<td>0.58</td>
<td>4.19</td>
<td>1.90</td>
<td>0.30</td>
</tr>
<tr>
<td>SPR 66 +TRP</td>
<td>3.05</td>
<td>0.49</td>
<td>4.53</td>
<td>1.88</td>
<td>0.24</td>
</tr>
<tr>
<td>SPR 83</td>
<td>3.14</td>
<td>0.67</td>
<td>3.35</td>
<td>2.08</td>
<td>0.24</td>
</tr>
<tr>
<td>SPR 83+TRP</td>
<td>3.19</td>
<td>0.44</td>
<td>4.78</td>
<td>2.09</td>
<td>0.26</td>
</tr>
<tr>
<td>SPR 100</td>
<td>3.12</td>
<td>0.49</td>
<td>4.36</td>
<td>2.84</td>
<td>0.26</td>
</tr>
<tr>
<td>SPR 100+TRP</td>
<td>3.49</td>
<td>0.61</td>
<td>4.96</td>
<td>2.37</td>
<td>0.30</td>
</tr>
<tr>
<td>HSD(0.05)</td>
<td>0.61</td>
<td>0.24</td>
<td>2.19</td>
<td>1.25</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Mean values with the same letter within column were not significantly different at \(p \leq 0.05\) according to Tukey’s studentized range (HSD) test.
to 6.51%. There was no significant difference in concentration of Ca and Mg in tissue with the inoculation and TRP treatments. The concentration of Ca ranged in between 1.88 to 2.84%, and Mg ranged between 0.23 to 0.30%.

The results showed that significantly higher values of chlorophyll content, total leaf area, concentration of N, P and K in tissue and the fresh weight of storage roots were observed on plants inoculated and added with selected rhizobacteria with addition of L-TRP. However, the number of sweetpotato storage roots was not influenced by rhizobacterial inoculation with or without addition of tryptophan. This indicates that the precursor is required for the bacteria to enhance the plant growth. The lowest plant growth was obtained from non-inoculated plant without TRP addition, indicating that presence of rhizobacteria is important in increasing plant growth and yield. Rhizobacteria can stimulate plant growth through several mechanisms such as increase in nutrient uptake, production of plant growth promoting substances and influence the rhizosphere’s environment (Kapulnik, 1991 and Kloepper, 1993).

The abilities of the rhizobacteria to effectively colonize root surfaces have a direct physiological effect on plant growth (Kapulnik, 1991). The root-colonizing bacteria could alter root cell permeability, which lead to increase ion uptake in plant. Earlier findings showed that increase in ion uptake could be due to increase in root surface area and not due to a specific acceleration of the ion uptake process (Okon et al., 1988). Usually, inoculum size has a marked effect on proliferation of root hairs at a specific root zone, and increase the potential surface area which may lead to increase nutrient uptake and root volume and finally increase the plant growth.

The presence of rhizobacteria in great numbers in the soil has an important role in many physiological processes and biochemical reactions for the growing plants. The most important effects are in carbon and nitrogen cycling which includes the biochemical decomposition and mineralization. Many, if not all, of these effects and reactions are related to other nutrient cycling (Tan, 1994). The bacteria also may improve plant nutrition by improving nutrient availability, enhancing plant uptake and by producing plant hormones especially IAA.

Inoculation of plant with the selected rhizobacteria increased the chlorophyll content of leaves, total leaf area and storage root initiation. This could probably be due to increase in nutrient uptake, especially N. Increase chlorophyll content of plant can increase the process of photosynthesis which will lead to increase in the total leaf area. Leaf area is a function of the total number of leaves and the size of leaves per plant (Kays, 1985), and has been utilized to measure the amount of photosynthetic activity.

The increased chlorophyll content, total leaf area and the storage root formation in this study could also be attributed to the effect of IAA producing rhizobacteria. Growth of plants applied with IAA was better than plants without IAA. The presence of endogenous hormones including gibberellic acid, auxin, ethylene, cytokinin and abscisic acid have been shown to improve plant growth and some physiological events (Kays, 1985). Previous study by Ewing (1987) reported that the initiation of storage root on root crop was stimulated by several plant hormones. Saad et al. (1996), have shown that inoculation of sweetpotato with PGPR Azospirillum increased growth and yield of sweetpotato. Azospirillum is known to produce plant-growth substances such as IAA and cytokinin in culture medium. The hormone might initiate reaction leading to longer-range of enzyme change that result in root growth and in morphogenesis (Dick and Tabatabai, 1993).

In general, the growth of plant was higher with addition of L-tryptophan (L-TRP) than plants without it. The concentration of IAA produced by rhizobacteria in soil was higher with L-TRP than without L-TRP addition. Isolate SPR 100 with addition of TRP had the best storage root yield compared to other treatments.
Effect of L-TRP on Performance of IAA (Maira):26 - 35
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Figure 3. Effect of Rhizobacterial Inoculation and L-TRP Addition on Population of Bacteria and Fungi in Soil. Mean values with the same letter were not significantly different at p ≤ 0.05 according to Tukey’s studentized range (HSD) test.

Population of Microorganisms in Soil

Soil inoculation with three selected rhizobacterial isolates and addition of TRP significantly (p ≤ 0.05) stimulated the bacterial population (Figure 3).

The populations of bacteria in soil inoculated with SPR 83 and SPR 100+L-TRP were significantly (p ≤ 0.05) higher than the non-inoculated soil without IAA (control 1). There was no significant difference in the population of fungi observed between the rhizobacterial inoculation and L-TRP treatments. The fungal population ranged between 3.73 and 4.26 log_{10} cfu g^{-1} soil.

Inoculation of soil with rhizobacterial isolates significantly influenced the population of bacteria but not the fungi. This could be due to the direct effect of the rhizobacterial inoculation. Several other factors such as plant, soil and microorganisms could also influence the microbial population in soil.

Plants influence microbial population by releasing plant substrates from root secretion or exudate. The root exudate or secretion contains various organic compounds such as amino acids, sugars, organic acids, proteins, polysaccharides, growth promoting and and activity of microbial population in the rhizosphere.

However, in the study only the total bacterial population was determined and not the different bacterial species. Addition of TRP, an amino acid seemed to stimulate the bacterial population in soil inoculated with SPR 100. The amino acid could provide energy and N source for proliferation of various bacterial species in soil.

Addition of L-TRP to the inoculated plants proved to increase the concentration of IAA and C-organic in soil. A similar result was observed by Frankenberger and Arshad (1995), where the auxin production was widespread among many soils and rhizosphere microorganisms, especially when L-TRP was added as a physiological precursor of auxin. In most soils, the low availability of L-TRP could be the most limiting factor in auxin production. Addition of L-TRP to soil could probably increase IAA production by the isolates, and may have physiological effects on plant growth and increased plant growth.

In this study, the addition of L-TRP on isolate SPR 83 decreased the population of bacteria in soil. It could probably be due to the presence of L-TRP which also increased growth inhibiting substances (Hale et al., 1978). The diversity of compounds present in root system of sweetpotato probably affect the composition the activity of soil pathogen.
survival of other microbes. Reinecke and Bandurski (1987), has found that the conversion of TRP to IAA is catalyzed by the plant pathogen *Pseudomonas savastanoi*. Increases of soil pathogen influence the activity and growth of endogenous microorganisms. Davies (1987) also reported the presence of L-TRP was found to negatively affect plant and several microbial growths. There also could be due SPR 83 had low tolerance to L-TRP applied into the soil.

The microbial population in soil could also be reduced by the presence of other indigenous microorganisms such as bacteria, fungi, or actinomycetes. It could be due to the negative interaction between soil microorganisms such as competition for nutrients and energy source. Some bacteria inhibited growth of other bacteria through production of antibiotics and siderophores (Klopper, 1993).

The Soil Nutrient Content

The rhizobacterial inoculation and L-TRP addition significantly (p ≤ 0.05) increased the concentration of IAA, but not the pH in soil (Figure 4).

The concentration of IAA in soils inoculated with three rhizobacterial isolates with or without L-TRP were and this condition could decrease the significantly (p ≤ 0.05) higher than both non-inoculated controls. Low IAA in control 2 indicated that addition of exogenous IAA could not sustain the IAA in soil as compared to soil inoculated with IAA producing rhizobacteria. The IAA concentration in soil inoculated with SPR 66 and SPR 100 with addition of TRP was significantly higher than that without TRP. However, addition of TRP to isolate SPR 83 showed a significantly lower IAA than that without TRP.

No significant difference in soil pH was observed between inoculation of rhizobacterial isolates and addition of TRP (Figure 4b). Addition of L-TRP to SPR 100 seemed to increase the pH in soil compared to that in control and other treatments. The pH of soils ranged between 4.96 to 5.51.

Inoculation with rhizobacterial isolates and addition of L-TRP significantly (p ≤ 0.01) increased concentrations of N and P, but not K, Ca and Mg in soil (Table 3).

The concentration of N in soil inoculated with SPR 100 with and without L-TRP addition was significantly higher than control (1). However, there was no significant effect of SPR 66 and SPR 83

![Figure 4](image-url)  
Figure 4. Effect of Rhizobacterial Isolates and L-TRP Addition on Concentration of IAA in Soil (a) and Soil pH (b). Mean values with the same letter were not significantly different at p≤ 0.05 according to Tukey’s studentized range (HSD) test.
or without L-TRP on the soil N. The concentrations of P in soil inoculated with SPR 66 with and without L-TRP, and SPRs 100 with L-TRP were significantly (p≤0.05) higher than control 1.

Rhizobacterial inoculation and addition of L-TRP into the soil significantly (p≤0.05) influenced the percentage of organic carbon and the ratio of carbon to nitrogen (C/N) in soil (Fig. 5). Addition of L-TRP to soil inoculated with SPR 100 significantly (p ≤ 0.05) increased percentage of soil organic C. However, addition of L-TRP to SPR 66 and SPR 83 showed no significant increase in organic C. The concentration of organic carbon in soil ranged between 0.58 to 0.95%. The concentration of soil organic carbon in non-inoculated soil without IAA (Control 1) was significantly lower than non-inoculated soil with IAA (Control 2).

The ratio of C and N (C/N) in the soil was significantly (p ≤ 0.05) influenced by rhizobacterial inoculation and addition of TRP. The C/N ratio in soil inoculated with SPR 66 and 83 with TRP addition was significantly higher than Control 1. Inoculation with rhizobacteria alone without L-TRP did not significantly affect the ratio of C and N in soil.

In general, the rhizobacterial isolates significantly increased the IAA, organic carbon, N, and P concentration of the soil but not the K and Mg. The increased IAA in the soil could be due to IAA synthesized by the inoculant. Addition of TRP further by the inoculant. Addition of TRP further increased the IAA in soil. The bacteria SPR 100 and SPR 66 probably synthesized IAA through TRP pathways (Reinecke and Bandurski, 1987).

The increase of P in soil might be influenced by plant and microbial interactions. The microorganisms could transform P through the transfer of inorganic to organic phosphate or transfer of phosphate from insoluble and immobilized forms to soluble or mobile compounds (Atlas and Bartha, 1987). The microorganisms also could produce organic phosphorus compounds as organic materials which are then transformed in soil like as phospholipids and nucleic acid (Mullen, 1998). Root excretions in the soils could also affect the P availability to plants (Bar-Yosef, 1991).

The increases of other elements such as nitrogen, carbon organic, and ratio C/N in the soils could be attributed to the effect of presence of microbial isolates and the organic compound. The increase in microbial population in soil could increase the soil biomass and increase the organic N. Increase N in soil also could be attributed to the effect of N2-fixation. N2-fixation is mediated exclusively by many genera of bacteria such as Rhizobium, Nostoc, Klebsiella or Frankia (Graham, 1998).

Increase concentration of organic C in soil could be contributed by plant residue. Plants contain variable concentration of protein, hemicellulose, cellulose and lignin
Figure 5. Effect of Rhizobacterial Isolates and L-TRP Addition on Soil Carbon Organic (a) and Ratio C and N (b). Mean values with the same letter were not significantly different at p≤0.05 according to Tukey's studentized range (HSD) test.

that can be quickly utilized by soil microorganisms and are important in microbial activity in rhizosphere (Paul and Clark, 1996).

In conclusion, the study showed that addition of L-tryptophan to the rhizobacterial isolates further enhanced plant growth and yield of sweetpotato. Improved growth and yield of sweetpotato with inoculation was related to the improved soil chemical properties and microbial activities.

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REFERENCES


effect of L-TRP on performance of IAA (Maira).


